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Review

Bacterial ureases in infectious diseases

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ABSTRACT – Ureases are multi-subunit, nickel-containing enzymes that catalyze the hydrolysis of urea to carbon dioxide and ammonia. This brief review discusses the biochemistry and genetics of bacterial ureases and outlines the roles of urea metabolism in microbial ecology and pathogenesis of some of the principle ureolytic species affecting human health. © 2000 Éditions scientifiques et médicales Elsevier SAS

Helicobacter / oral microbes / ecology / pathogenesis / urinary tract

1. Purpose

The economical and medical significance of bacterial ureases has been realized for many years, but more widespread interest in microbial ureases has been stimulated by such relatively recent events as the discovery of the association of *Helicobacter pylori* with gastritis and stomach cancers, the application of molecular genetic tools to the study of urease genes and enzymes, and the recognition of the fascinating complexities of urease gene regulation and enzyme biogenesis. Within the past decade, a number of very informative and comprehensive review articles have been written about microbial ureases and about the pathogenic bacteria which produce urease. The purpose of this communication is to give a brief overview of why bacterial ureases are important factors in infectious diseases and to highlight some more recent developments that have occurred as a result of the application of molecular techniques to the study of ureolytic bacteria.

2. Biochemistry and genetics of bacterial urease enzymes

2.1. Urea metabolism

Urea is the major nitrogenous waste product of most terrestrial animals. Urea is produced in the liver, carried in the bloodstream to the kidneys, and excreted in urine. Serum concentrations of urea in healthy humans are between one and 10 mM, but urea levels in urine can exceed 0.5 M [1]. Urea is also present in the secretions of the major and minor exocrine glands at concentrations approximately equivalent to serum, so a large proportion of circulating urea is translocated onto epithelial surfaces by secretory systems, or in tissue exudates. For example, adult humans secrete almost 1 liter of saliva per day containing 1–10 mM urea [2], and some 20–25% of all urea produced enters the intestinal tract rather than exiting the body in urine [3]. There does not appear to be active efflux mechanisms for exocrine secretion of urea, so it is believed that the uncharged urea molecule simply follows water through the cells and tight junctions of the epithelium. As a consequence, the epithelial surfaces of the human body are bathed in a fluid which contains urea [4]. On sterile tissues urea is a relatively benign component, playing no apparent role in maintenance of tissue integrity or other homeostatic processes. In contrast, in sites where microorganisms colonize epithelial surfaces, such as the normal flora of the oral cavity or intestines, or when certain pathogenic bacteria establish on tissues, the metabolism of urea by microbial ureases can have a profound impact on tissue integrity, microbial ecology, and the overall health of the host.

2.2. Microbial ureases: gene organization, structure and biogenesis

A wide variety of environmentally and medically important bacteria produce the enzyme urease (urea amidohydrolase; E.C.3.5.1.5) [5–7], which catalyzes the hydrolysis of urea, leading to the production of carbamate and ammonia *(figure 1)*. In an aqueous environment, the carbamate rapidly and spontaneously decomposes to yield a second molecule of ammonia and one of carbon dioxide. A number of recent studies have provided detailed information about the genetics of ureases from a variety of evolutionarily diverse bacteria, plants and fungi, and these have been conveniently compiled and summarized in recent review articles [5, 7]. Considering the comparatively simple reaction performed by the enzyme, ureases are structurally complex and, unlike most enzymes, addi-

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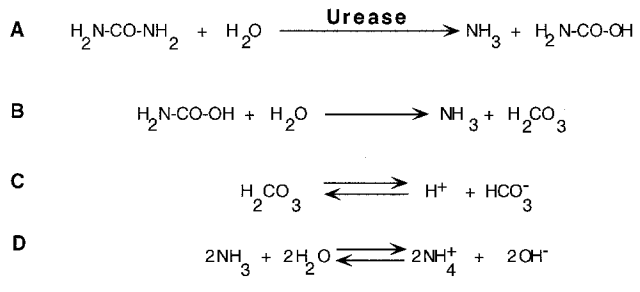

Figure 1. The urease reaction. Urea is cleaved by urease to produce one molecule of ammonia and one of carbamate (A). Carbamate spontaneously decomposes to ammonia and carbonic acid (B). The carbonic acid equilibrates in water (C), as do the two molecules of ammonia, which become protonated to yield ammonium and hydroxide ions (D). The reaction results in a rise in the pH of the reaction environment.

tional urease specific gene products are required for the production of a catalytically active holoenzyme. Most bacterial urease gene clusters contain the *ureABC* genes, which encode the γ, β and α subunits, respectively, that form the urease apoenzyme. Some variations in gene arrangement and number of subunits occur between bacteria, but the subunits of bacterial ureases can be linearly aligned with one another and with the single-subunit urease isolated from jack bean [8]. For example, the gastroduodenal pathogen, *H. pylori* ureAB genes are sufficient to encode urease [9, 10], but the UreAB subunits of this organism can be aligned with the UreABC subunits of other ureolytic bacteria and with the single polypeptide of the jack bean urease. The crystal structure of the *Klebsiella aerogenes* urease has been solved and the enzyme has been shown to be assembled in a trimeric (α, β, γ)3 configuration [11]. Biochemical analyses of ureases by gel filtration have shown that other bacterial ureases are multimeric, and probably have similar stoichiometry [6]. Native gel electrophoresis followed by activity staining of urease enzymes also reveals that multiple isoforms of urease commonly exist in bacteria, although the molecular basis for the existence of multiple bands is not established.

Ureases are among the few enzymes that require nickel for activity, and in the vast majority of cases other metals cannot replace nickel to yield an enzyme with comparable biological activity. Biogenesis of a functional urease requires the presence and expression of four urease accessory genes, *ureDEFG*. In vitro experiments using purified accessory proteins support that UreE likely acts as a carrier of nickel [12] and that UreDFG form a chaperone-like complex that keeps the apoenzyme in a configuration competent to accept nickel [13]. Carbamylation of the enzyme is also required for efficient and productive nickel incorporation [14], perhaps explaining the enhancement of urease activity when certain organisms are grown in a CO_2-rich environment. It is also interesting that the enzyme produces CO_2 as a product. In addition to the accessory genes, other genes are involved in urease biogenesis. High-affinity metal transporters, such as the *nixA* gene of *H. pylori* [15], scavenge the trace element nickel from the environment. There are also genes which are associated with *ure* gene clusters, e.g., *ureI* of *Bacillus* sp. [16], *Helicobacter* sp. [9] and *Streptococcus salivarius* [17]. In some cases, ureI has been shown to be cotranscribed as part of the urease operon [18], but the function of UreI is not defined. Interestingly, many ureolytic species display an unstable urease phenotype after initial isolation and in vitro passage, and this is true whether the genes are plasmid-borne, as in some enterococci, some strains of *Escherichia coli* and *Providencia stuartii* [4], or chromosomally located, as in some staphylococci and streptococci [19, 20]. Therefore, not only is the generation of a functional urease a complex process, but there must be significant evolutionary pressure on some organisms to maintain apparently unstable genes in vivo.

2.3. Regulation of urease gene expression

The ability to differentially regulate urease may be a critical factor in the contribution of the enzyme to colonization, persistence and pathogenesis. In particular, ureolytic organisms are sensitive in vitro to physiologically relevant concentrations of urea, since they can rapidly drive the pH to levels greater than nine. Therefore, downregulation of urease may be important to survival in certain environments. Differential regulation of urease may also be important because many overt and opportunistic pathogens can occupy multiple niches outside and inside the human body, and activation of urease only when it is needed would enhance competitive fitness of the bacteria. In many cases, the expression of bacterial ureases is tightly regulated, usually by activation of urease transcription under nitrogen-limiting conditions or by induction by urea [5, 7]. It is also widely believed that a variety of ureases are expressed constitutively by bacteria, although many of these studies have not looked exhaustively at environmental variables that might influence urease gene expression beyond nitrogen availability and urea.

Detailed molecular studies to determine the basis of regulated expression of urease genes have been confined to only a few organisms. Urease expression by *Proteus mirabilis* is inducible by urea. Induction is mediated through the positive transcriptional regulator UreR [21], which binds nearby to the promoter of the *ure* genes [22]. In contrast, the urease genes of *Klebsiella pneumoniae* are activated through a two-component signal transduction system that globally regulates gene expression in response to nitrogen availability [23, 24]. A novel form of urease gene regulation has been described in the oral bacterium *S. salivarius* [25–27]. The urease genes of this organism are arranged in an operon and are transcribed from a sigma 70 type promoter [18]. Urea or nitrogen availability do not seem to affect urease expression to any great extent, whereas growth at neutral pH values results in nearly complete repression of transcription [27]. Repression is exerted primarily by binding of an as yet unidentified transcription factor(s) to a region of the DNA located immediately upstream of the –35 element of the urease promoter [18]. The operon becomes derepressed at acidic pH values, especially below pH 6.0, and once derepressed, the level of expression is further increased when

cells are growing under carbohydrate excess conditions. Control by carbohydrate availability is governed in part through the bacterial sugar:phosphotransferase system [28]. Induction by low pH may represent an acid adaptive response which allows the cells to alkalinize the surroundings and the cytoplasm through the production of ammonia. Consistent with this idea is the fact that, unlike wild-type *S. salivarius*, an otherwise isogenic, urease-deficient mutant is not protected by urea from lethal acidification by the presence of urea (Chen et al., unpublished). Effects of environmental pH on urease expression in non-oral bacteria have not been explored in as great detail as with the oral bacteria, but there is some indication that pH may regulate urease gene expression in *Helicobacter* [29] and *Proteus* [30], albeit to a lesser degree than in streptococci.

Relatively little information is available on the transcriptional organization of urease gene clusters. In those cases that have been documented, some or all of the urease genes can be cotranscribed from a proximal promoter(s) [5, 7]. Of note, after the accessory gene products have activated the urease enzyme, they presumably can dissociate to bind and activate a newly synthesized apoenzyme [13]. Therefore, the amount of the ureDEFG gene products required by the cell may be substantially lower than for the structural subunits, ureABC. Recently [18], one mechanism for achieving higher levels of mRNA for the structural genes has been proposed for *S. salivarius* and may involve preferential termination at an internal transcription terminator located immediately downstream of *ureABC* and just upstream of *ureEFGD*.

3. Role of bacterial ureases in pathogenesis

3.1. Role of ureases in microbial virulence

Many recent studies that have used in vivo expression technology or signature tagged mutagenesis to identify virulence determinants of bacteria have highlighted the importance of intermediary metabolism in the ability of pathogens to persist in vivo and to cause disease, presumably because certain metabolic pathways are required to synthesize compounds that are absent or sequestered in the host. Considering the reaction that is catalyzed by urease, it would be logical to assume that the enzyme is involved primarily in nitrogen assimilation. In fact, urea does represent an assimilable nitrogen source for bacteria that can colonize the human body and there is evidence suggesting that ammonia assimilation from urea occurs in vivo. For example, a significant proportion of the urea produced in the liver ends up in the intestines, where it can be hydrolyzed and assimilated by several different species of anaerobic, ureolytic bacteria [4]. Similarly, the abundant oral bacteria, *S. salivarius* (Chen et al., unpublished) and *Actinomyces naeslundii* [31], can use urea as a primary nitrogen source for growth. So there is little doubt that nitrogen acquisition from urease may be important in the ecology of complex populations colonizing the human body. However, it is questionable whether the conferring of the capacity to assimilate urea nitrogen by urease is a contributor to the pathogenic potential of bacteria. Instead, it appears that the release by urease of the strongly alkaline agent ammonia is the major cause of the damage to the host tissue, and in some cases, a key factor in persistence of pathogens.

In most organisms, urease enzymes are found in the cytoplasm, and there are numerous reports of membrane association and cell surface localization as well [6]. The ureases for which primary sequence information is available do not have characteristics consistent with being integral membrane proteins or secreted through the general secretory pathways. Urea, particularly at millimolar concentrations and above, can readily enter the cell by diffusion, although some evidence has been presented for active transporters in a variety of organisms [6] which probably function at low urea availability. Once urea is cleaved, there are a few possible routes for the products to take *(figure 2)*. In one case, the ammonia (pKa = 9.2) can become protonated to produce ammonium ion, which has the effect of alkalinizing the cytoplasm and contributing to the ΔpH component of the membrane potential [29, 32, 33]. The uncharged ammonia molecule can also diffuse from the cell where it can drive the pH of the extracellular environment into the alkaline range. *Figure 3* shows a pH versus time plot of the ureolytic oral bacterium simultaneously metabolizing glucose and physiologically relevant levels of urea [17]. In this case, with urea alone, the pH is driven to a value near the pKa of ammonia (9.2), but ureolysis can also significantly blunt glycolytic acidification. Release of carbon dioxide from urea also influences bacterial pH homeostasis, bacterial physiology and the environmental pH, as well as the buffering capacity of the biofilms in which the infecting organisms are enmeshed, but ammonia release appears to have the greatest impact in terms of pathogenesis. It should also be noted that the urinary tract pathogen *Ureaplasma ureolyticum* [34] and some alkalophilic bacteria [35] can use ureolysis to generate ATP.

The impact of the release of copious quantities of ammonia on the surrounding tissues varies with the site and the organism. Some of the problems caused by ureolysis are due directly to pH effects, whereas other pathologies arise as a result of the direct toxicity of ammonia derivatives. For example, the functioning of mammalian ion pumps, receptor:ligand binding activity, enzyme activity and the integrity of the protective films coating epithelial surfaces can be adversely affected at pH values that are elevated significantly above what is normal for a particular site. Ureolysis can also lead to the formation of ammonium hydroxide, which is highly toxic to mammalian cells. Another route for cell damage has been proposed when urease or ureolytic bacteria interact with leukocytes. The formation of ammonia in the presence of the oxidative burst created by immune cells can lead to the formation of monochloramine [36], which has been shown to be able to induce DNA damage. In addition to the direct deleterious effects of ammonia formation, the capacity of the immune system to combat infection may also be compromised because of the inhibitory effects of elevated pH and ammoniacal compounds on immune function.

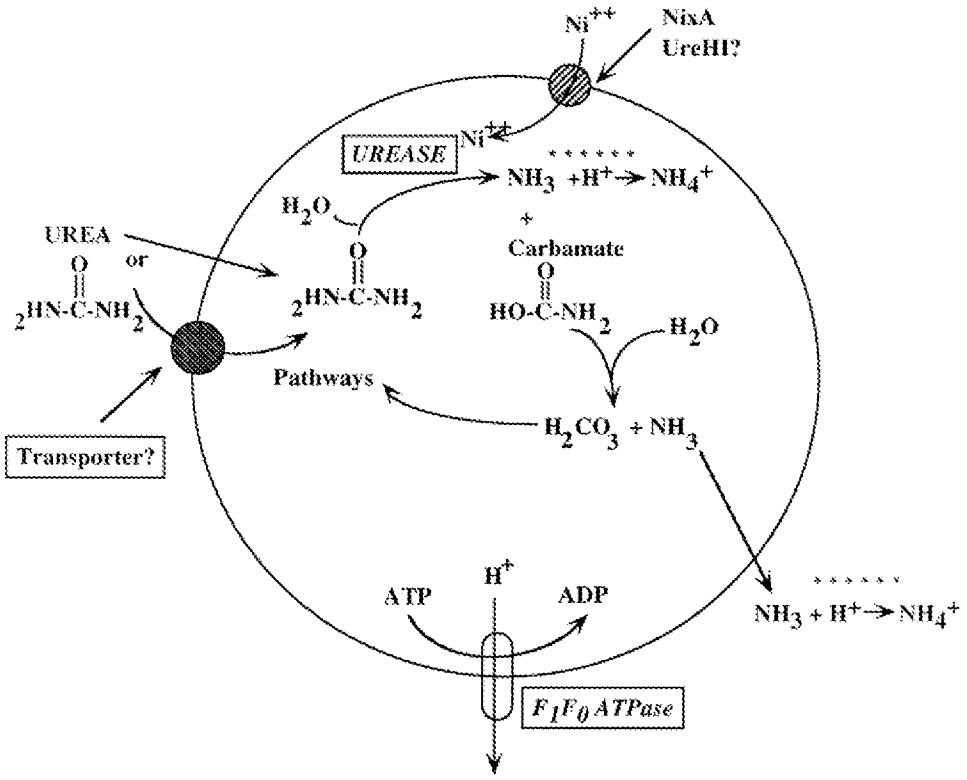

Figure 2. Pathway for urea metabolism and effects on cytoplasmic and environmental pH. Urea can enter the cells by simple diffusion, although urea transporters have been identified in some organisms. Inside the cell, urea is cleaved to yield ammonia and carbon dioxide. As indicated in *figure 1*, the ammonia inside the cell can become protonated with a net increase in cytoplasmic pH, which can help to maintain DpH. In some organisms, such as the *S. salivarius*, this is a major advantage because the organisms rely almost solely on the F-ATPase to extrude protons in order to maintain membrane potential. The uncharged ammonia molecule can also rapidly diffuse across the cell membrane and raise the extracellular pH. Ammonia from urea can also be assimilated as a nitrogen source and the released CO_2 could also affect the physiology of the organisms by entering anabolic pathways. In the case of *H. pylori* and perhaps other organisms, urease which can somehow become associated with the cell surface, is an important factor in survival in severely acidified environments, probably because neutralization of the extracellular milieu around the cells is needed to prevent irreversible membrane damage. Asterisks highlight the key factors in pH modulation and host toxicity.

3.2. Urease and *Helicobacter* infections

H. pylori can be isolated from the human stomach and duodenum. Formerly know as *Campylobacter pylori*, the organism is a spiral shaped, Gram-negative bacterium that is a causative agent of gastritis and gastroduodenal ulcers. Chronic infection with *H. pylori* appears to be a risk factor for the development of adenocarcinomas and gastric lymphomas. For a long time, the stomach was believed to be too hostile an environment for persistent colonization by bacteria because the pH is well below that which will support growth of most microorganisms. However, the production of a potent urease by *Helicobacter* allows this organism to persistently colonize the gut, and ureolysis is a major contributor to the elicitation of the pathologies associated with *Helicobacter* infection.

Normally, *H. pylori* grows in a rather narrow pH range, around 6–8 [37], and is rapidly killed at pH values below 4. Its ability to persist in the stomach is very much dependent on the production of, among other factors [38, 39], high amounts of urease activity [40, 41]. Convincing evidence has emerged that the urease of this organism can be found both in the cytoplasm and on the cell surface [42], although it appears that cultivation of *H. pylori* under certain growth conditions results in substantial amounts of the enzyme being localized to the cell surface [43]. Thus, organisms growing in the gut appear to have both an intracellular urease and significant amounts of active enzyme coating the cell surface. The current view of the connection between ureolysis and growth of *Helicobacter* in low pH environments is that urea metabolism creates a microenvironment which is in a pH range that will allow for multiplication of the bacteria. *H. pylori* is motile in viscous environments and can penetrate the mucous layer lining the gastric epithelium, where the bacterium can adhere using multiple mechanisms [38, 39]. The urease of this organism hydrolyzes the urea that is present in gastric secretions and exudates, creating a much more pH neutral environment that can support the growth of the organism [44]. Results from in vitro experiments suggest that the extracellular form(s) of urease is essential for protection from acid killing [45], presumably because neutralization of the surroundings prevents lethal membrane damage.

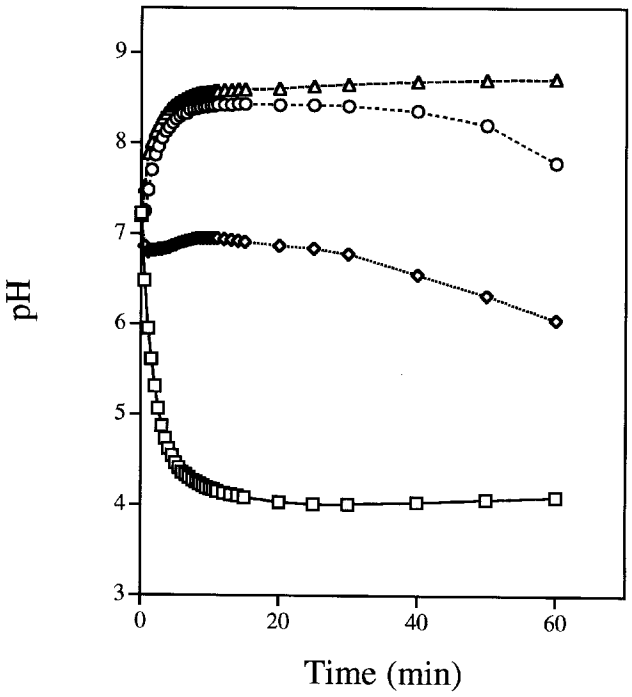

Figure 3. pH versus time plot of recombinant ureolytic Streptococcus gordonii. A previously described strain of S. gordonii [17] which carries the urease genes of *S. salivarius* was grown under conditions which allow for high expression of urease. The cells were then presented with 55.6 mM glucose and 0 (square), 2 (diamonds), 5 (circles), or 10 (triangles) mM urea.

Ureolysis also contributes to the capacity of *H. pylori* to persist by helping the organisms to maintain ΔpH [32, 33]. Importantly, the consequences of urease production by *H. pylori* appear to extend far beyond the single finding that they protect the bacteria from being killed in a strongly acidic site. As indicated above, the generation of ammonia, which has little effect on the metabolic activity and growth of bacteria, can have a profound effect on the host tissue. Ammonia release by urease has been shown to have a direct cytotoxic effect on gastric epithelial cells [46]. Moreover, the formation of monochloramine can occur when leukocytes, urea and *Helicobacter* urease are present in the same reaction [36]. Monochloramine can induce mutagenic DNA damage in the host [47] and is believed to be a contributing factor to the development of cancers associated with chronic *H. pylori* infection.

3.3. *H. pylori* urease and the immune response

The urease of *H. pylori* is a major antigen recognized by the host during infection; not surprisingly given both the abundance of the protein in the organisms, as well as the potential for localization of urease on the cell surface. In infected individuals, elevated circulating and secretory immunoglobulin levels against *H. pylori* urease are common [48], as is a cell-mediated immune response to urease and *Helicobacter* [49]. *Helicobacter* infection is believed to be amazingly widespread and estimates of the number of individuals harboring the organisms have approached 50% in some populations [50]. Treatment and elimination of the organism can be expensive, time consuming and difficult, reinfection can occur rapidly, and concerns about development of multidrug resistance are certainly warranted. Consequently, numerous studies have focused on urease as a vaccine candidate [51].

An early vaccine study attempted to determine if the immunologically cross-reactive jack bean urease could be used as an antigen, but the plant enzyme did not prove particularly effective in eliciting cross-protection [52]. Subsequently, killed *Helicobacter* cells were used with a rodent model in which the challenge organism was *Helicobacter felis*, which is closely related to *H. pylori*. Moderate success was realized, but more recently, the use of highly purified, recombinant urease subunits has proven to be a much more effective strategy. Initially, mice were infected with H. *felis*, and three weeks later were orally immunized with a purified urease preparation. Within 3 to 8 weeks, the infection resolved and the mice remained resistant to subsequent challenge with H. *felis* [53]. Vaccine studies have been expanded to include the use of nonhuman primates [54]. In general, recombinant urease, given orally and in conjunction with heat-labile enterotoxin in several doses can elicit a strong IgG and IgA response. Animals which were vaccinated with *H. pylori* urease were protected to a statistically significant degree [55, 56], and protection appeared to be long term [56]. Studies with oral administration of *H. pylori* urease to human volunteers have begun and the vaccine appears to elicit a strong immune response and to have minimal side effects [57]. The efficacy of these preparations in protecting humans from subsequent infection or in resolving existing infections is unknown.

3.4. Bacterial urease and urinary tract infection

Urine from healthy humans consists of a large quantity of nitrogenous compounds, including 0.5 M urea, as well as inorganic ions. Urine is neutral to slightly acidic, and under these conditions, ammonia becomes protonated with the concomitant generation of hydroxide, which increases urine pH. The alkaline pH causes the precipitation of normally soluble polyvalent cations and anions in urine, leading to the formation of urinary stones. The major urinary stones are composed of struvite ($MgNH_4PO_4 \cdot 6H_2O$) and carbonate apatite [$Ca_{10}(PO_4)6CO_3$] [4, 5]. The most common ureolytic species isolated from the urinary tract are Enterobacteriaciae, in particular *P. mirabilis*. Other species include *Pseudomonas* spp., *Klebsiella* spp., *Staphylococcus* spp., *Corynebacterium* spp., *U. ureolyticum* and *Proteus penneri*. Ureolytic bacteria in general are not the major cause of urinary tract infection in healthy individuals, but are frequently associated with infections in patients with urinary tract complications [5]. In earlier studies using an in vitro artificial urine system, it was demonstrated that ureolytic microorganisms or jack bean ureases could elevate the pH of urine, which led to the formation of struvite and carbonate apatite [58]. Neither nonureolytic microorganisms, nor ureolytic microorganisms treated with the urease-specific inhibitor acetohydroxamic acid (AHA), were able to increase the pH of urine. On the other hand, chemically

increasing the pH of the artificial urine with NH_4OH or NaOH resulted in the formation of carbonate apatite, and ammonia-induced pH elevation was essential for the production of struvite. The impact of urease in induction of pyelonephritis caused by *P. mirabilis* also has been evaluated in animal models. Earlier results using a chemically mutagenized urease-negative strain [59], or using acetone-killed cells that retained urease activity [60], suggested that ureases play a central role in the development of renal pathology. These conclusions were further confirmed in studies done by treating *Proteus*-infected rats with AHA [61]. Although comparable numbers of bacteria were detected in urine of both rats treated with AHA or receiving no treatment, fewer bacteria were isolated from the kidney and less tissue damage was observed in rats treated with AHA. Furthermore, no common enterobacterial antigens could be detected in rats treated with AHA, indicating that urease may play an important function in invasion. The urease-dependent invasive property of *P. mirabilis* is supported by in vitro observation that urease, in conjunction with hemolysin activity of this organism, may contribute to the cytotoxicity for cultured human renal proximal tubular epithelial cells. Thus, it is likely that alkalinization by ureolysis has deleterious effects on renal epithelium and enhances the severity of pyelonephritis [62]. More recently, the contribution of urease to the virulence of *P. mirabilis* was evaluated in a CBA mouse model by a mutant strain in which the gene encoding the major subunit of urease was insertionally inactivated [63, 64]. Animals were infected transurethrally, which mimics the route of natural infection. In this case, the wild-type strain was able to establish in the urine, bladder, and kidney 48 hours postinfection, but not the mutant, suggesting that urease plays a significant role in colonization of the urinary tract. Urease-deficient strains exhibited an ID_{50} of 2.7×10^9 CFU, which was more than 1 000-fold higher than the wild-type strain (2.2×10^6 CFU), and only the wild-type strain was able to persist significantly. The severity of histopathological lesions in kidney increased over a 2-week period postinfection in animals infected with wild-type bacteria, and severe renal damage was observed in 6 out of 19 mice. In contrast, animals challenged with urease-negative mutants only developed mild to moderate pyelitis, and the majority of the animals (12 out of 18) never developed kidney lesion. Urolithiasis was only found in animals infected with wild-type organisms, and the analysis of the stones revealed struvite. In addition, it has been demonstrated recently that the polysaccharide capsule (CPS) of *P. mirabilis* enhances struvite formation, especially at moderately alkaline pH values, under which conditions CPS shows a higher binding capacity for Mg^{2+}. Thus, it is suggested that CPS might weakly concentrate Mg^{2+} during crystal formation [65], although urease is probably essential for effective formation because of the need for an alkaline environment.

Unlike *P. mirabilis*, *Staphylococcus saprophyticus* is a frequent cause of urinary tract infections only in young female adults. The contribution of urease to the pathogenicity of this organism has been demonstrated in a rat model using a chemically mutagenized, urease-deficient strain. Urinary stones were found only in animals infected with wild-type bacteria but not with the mutant strain, and only sera from animals infected with wild-type bacteria reacted with many antigens of *S. saprophyticus*. In contrast, only very weak responses were detected with sera from animals infected with the urease-negative mutant [66]. When the urease gene cluster was expressed in a nonureolytic staphylococcal strain, the recombinant ureolytic strain was able to establish infection and to develop inflammation in the bladder as effectively as the wild-type cells, indicating that urease plays a major role in the cytopathogenicity [67]. Thus, the role of urease in urinary tract infection and stone formation is well established.

3.5. Urease in other bacterial diseases

A variety of other human and animal pathogens express urease, and in many cases, ureolysis is thought to contribute to the infectivity or persistence of the organisms, or to the pathologies of the disease. Space constraints prevent a detailed description of these infections, but there are a few ureolytic pathogens worth highlighting here. *U. urealyticum* is a relatively infrequent, but important causative agent in urinary tract infections and can be a serious cause of infections of the respiratory tract. As indicated above, ureolysis can be used by *U. urealyticum* for generation of membrane potential to drive ATP synthesis [34]. The contribution of urease to *U. urealyticum* pathogenesis in the urinary tract is thought to be similar to that of other urinary tract pathogens, but the role of urease in *Ureaplasma* virulence of either urinary or respiratory infections is largely unexplored [5]. The use of a urease-deficient mutant of *Bordetella bronchiseptica*, however, has shown that urease is not essential for pathogenesis in a guinea pig model [68]. Also, *Yersinia enterocolitica* produces a urease enzyme. Interestingly, certain individuals have a genetic predisposition to develop reactive arthritis following *Y. enterocolitica* infection which is apparently attributable to reactivity with the UreB subunit [69]. Finally, a number of bacteria responsible for infection of in-dwelling devices can be ureolytic, and the formation of biofilms and mineral deposition on catheters and other medical devices may be exacerbated by urease production [4].

3.6. Ureases in the oral cavity

Urea is present in secretions of the major (parotid, sublingual and submaxillary) glands, in the minor salivary glands, and in the exudate known as gingival crevicular fluid [2], which seeps into the mouth between the gum and teeth. Generally, the concentration of urea entering the mouth is about the same as serum, 3–10 mM, and the average human produces about 1 L of saliva per day. The ureolytic activity of saliva, which contains about 10^6 organisms per ml, and of dental plaque is usually around 1 μmole min^{-1} mg^{-1} wet weight [70, 72], so the urea which enters the mouth is hydrolyzed rapidly. Interestingly, there remains some questions about which organisms are responsible for the bulk of ureolysis in the oral cavity. *S. salivarius* is an abundant colonizer of the soft tissues, including the tongue, and can produce as much as 5 units of urease per mg dry weight of cells in vitro [19, 27], roughly corresponding to about 2.5 U mg^{-1} wet weight. This organism is probably the major contributor to ureoly-

sis on soft tissues and in saliva. However, *S. salivarius* is not a significant constituent of tooth biofilms, where the primary ureolytic bacteria are *Actinomyces* and haemophili [73].

It is believed that ureases may play major roles in maintenance of oral health and perhaps in oral diseases as well. Dental caries, which afflicts the vast majority of the population, is caused by fermentation of dietary carbohydrates by acid-tolerant organisms, such as *Streptococcus mutans* and *Lactobacillus* casei, which increase in proportions in cariogenic tooth biofilms. Repeated cycles of plaque acidification can result in dissolution of tooth enamel leading to formation of dental caries, i.e. cavities. Urea metabolism can counteract glycolytic acidification of dental plaque [70, 71, 74, 75] and is believed to be a major factor in dental plaque pH homeostasis. A variety of data support this notion, including the finding that chronic renal failure patients [76], who can have salivary urea levels around 50–80 mM prior to dialysis, develop essentially no caries in spite of consuming a diet consisting of mostly carbohydrates. The ability of urea and urea-containing rinses to increase dental plaque pH and to promote enamel remineralization has been demonstrated [77]. More recently, by introducing the urease genes of *S. salivarius* into a nonureolytic, cariogenic streptococcus, *S. mutans*, it was shown that physiologically relevant levels of urea could prevent a pH fall in vitro by cells metabolizing as much as a 100-fold molar excess of glucose, provided the cells produced sufficient levels of urease enzyme [78]. Thus, it was proposed that not only was caries dependent on increases in the proportions of acidogenic and aciduric species, but that perhaps a diminution of alkali-generating capacity from loss of the less acid-tolerant, ureolytic organisms contributed to the development of the disease. Preliminary efforts to test this hypothesis in an animal model support this concept [79].

There is indirect evidence that urea metabolism may contribute to other common oral maladies, including the formation of calculus and the development of periodontal diseases. Calculus, or tartar, forms when minerals – primarily calcium, phosphate and magnesium – precipitate onto the tooth surface. This process, like stone formation in the urinary tract, is encouraged by increases of the pH significantly above 7.0. No formal connection has been made between ureolysis and calculus formation, although end-stage renal dialysis patients who have very high levels of urea in their saliva form tremendous amounts of calculus [80] and calculus can contain significant proportions of struvite [81].

The connection between periodontal diseases and ureolysis is also largely unexplored, but circumstantial evidence would indicate that the ammonia released from urea may contribute to the tissue damage that is characteristic of these diseases. Virtually all individuals will experience some form of periodontal disease in their lifetime. Periodontal diseases are inflammatory diseases that result when there is an upset in the ecologic balance of the organisms colonizing portions of the tooth and the tissues below the gum line, resulting in a marked increase in the number of Gram-negative anaerobic bacteria. If left untreated, periodontal diseases can lead to considerable loss of attachment of the connective tissue to the tooth, bone loss, and ultimately to loss of the tooth. Urea can enter the gingival crevice from saliva or from the serum exudate bathing the periodontal pocket [2]. Measurements of urea in the gingival crevice of healthy individuals are similar to those found in salivary secretions, suggesting that ureolytic organisms are not present in substantial numbers. However, when the periodontal pocket becomes inflamed as in the disease state, detectable urea levels drop precipitously, even though more urea is entering the area, as part of the inflammatory process [2, 82]. Concurrently, there is a proportionate increase in the amount of ammonia detected in the region and the pH is significantly elevated, suggesting that the urea is being metabolized rapidly by oral bacteria in the region. Oddly, an informal survey of over 300 species isolable from the inflamed pockets of periodontitis patients does not reveal any obvious increases in the numbers of ureolytic organisms (Burne, unpublished). Thus, the microbiological basis for decreased urea in periodontal pockets with evidence of disease remains to be established. Still, as with other epithelial surfaces, direct toxicity to fibroblasts and immune cells exposed to elevated levels of ammonia and its derivatives contributes to the tissue loss and inflammation in periodontal diseases [83]. Interestingly, the increase in pH from ureolysis can enhance the activity of several bacterial and host proteases, including collagenase [82], and thus may enhance disease progression in that manner. One of the major clinical problems in periodontal disease is the formation of calculus, which may be promoted by the alkaline pH environment created by urea metabolism. Clearly, more work needs to be done in this area to illustrate whether therapies aimed at controlling urea breakdown could be beneficial to periodontitis patients.

4. Conclusion

Adult humans excrete over 20 pounds of urea per year. Much of the urea that is produced is rapidly metabolized to ammonia by the resident flora, such as in intestines and the oral cavity. Urea metabolism plays major roles in the ecology of the complex microbial populations constituting the normal flora of the human. The resident flora can be strongly protective against colonization or emergence of overt pathogens at many sites in and on the body. Maintenance of the pH in complex populations and the competitive edge that may be gained by bacteria that can assimilate nitrogen through a urease-dependent pathway may help to stabilize a flora that is compatible with health and help to prevent invasion or emergence of pathogenic species. Conversely, excessive ammonia released by pathogenic, ureolytic organisms can have a profoundly negative impact on human health. Much valuable basic and applied information has been generated as a result of studies of ureases, and it is certain that additional new and exciting discoveries will arise as work on the genetics, biochemistry, ecology and pathogenesis of ureolytic bacteria continues.

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